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Short Communication

Matrix-assisted laser desorption ionization-time of flight: a promising alternative method of identifying the major coagulase-negative Staphylococci species

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Abstract

Introduction. This study aimed to evaluate different methods for differentiation of species of coagulase-negative staphylococci (CoNS) that caused infections in hospitalized immunocompromised patients. **Methods.** A total of 134 CoNS strains were characterized using four different methods. **Results.** The results of matrix assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS) analysis were in complete agreement with those of *tuf* gene sequencing (kappa index = 1.00). The kappa index of Vitek 2® Compact analysis was 0.85 (very good) and that of the conventional method was 0.63 (moderate). **Conclusions**. MALDI-TOF MS provided rapid and accurate results for the identification of CoNS (134; 100%).

Keywords: Coagulase-negative staphylococci. MALDI-TOF. *tuf* gene.

Coagulase-negative staphylococci (CoNS) constitute an important group of opportunistic pathogens that cause infections predominantly in hospitalized immunocompromised patients, which are associated with indwelling or implanted medical devices. The species most frequently associated with human infections are *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, and *Staphylococcus saprophyticus*, followed by *Staphylococcus hominis*, *Staphylococcus warneri*, *Staphylococcus capitis*, *Staphylococcus simulans*, and *Staphylococcus lugdunensis*; most isolates are resistant to multiple drugs[1,2]. The increasing incidence of CoNS hospital-acquired infections emphasizes the need for accurate and rapid identification of *Staphylococcus* at the species level. Although the ability to distinguish between these pathogens is clinically relevant, a number of laboratory limitations still exist[1,3]. Molecular methods are currently favored for accurate identification and several target genes, such as *tuf* gene, exhibit high discriminatory power[4]. The aim of this study was to compare the ability of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (Microflex LT instrument, Bruker Daltonics, Bremen, Germany) and three other identification methods to differentiate between CoNS samples.

A total of 134 CoNS clinical isolates from the blood cultures (112) and catheter tips (22) of patients hospitalized in a tertiary care teaching Hospital in Curitiba, Brazil were studied. The discriminatory power of MALDI-TOF MS was compared to that of the gold standard *tuf* gene sequencing method, an automated method (Vitek® 2 Compact, bioMérieux, Marcy l'Etoile, France), and a conventional method for all CoNS included in the study. In addition, the minimum inhibitory concentrations (MICs) of the antimicrobials oxacillin, daptomycin, linezolid, and vancomycin were evaluated and the presence of the *mecA* gene was determined by PCR for all strains.

All bacterial isolates were preliminarily identified by catalase, coagulase, and deoxyribonuclease tests. In addition, the disk diffusion assay was used to determine susceptibility to fosfomycin (200µg) and desferrioxamine (300µg)[5]. Subsequently, the strains were evaluated using the conventional method proposed by Cunha et al., for distinguishing between species not identified by the previous tests[3]. *S. epidermidis* ATCC 12228 was used as the control strain. Finally, the Vitek® 2 Compact automated system was used as per the manufacturer's instructions; the acceptable criterion for identification was set at 93-99% probability.

For MALDI-TOF MS analysis, a loopful of cells from a fresh overnight pure culture was processed as previously described[6]. The obtained mass spectra were analyzed using the Microflex LT instrument with MALDI Biotyper 3.0 software. The identification score cutoff value was set at ≥1.8[7].

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For sequencing of the *tuf* gene, template DNA was extracted from all 134 CoNS and a reference strain (*S. epidermidis* ATCC 35984) using the post-boiling (15 min at 95°C) technique. The reaction mixture, cycling conditions, and primer sets used for PCR were those previously described by Hwang *et al*[8]. All products (412bp) were purified using the EXO SAP kit (Amershan Science, USB, Cleveland, OH, USA) and sequenced with the ABI Prism™ BigDye Terminator kit (Perkin-Elmer, Foster City, CA, USA), according to the manufacturer's protocol. Forward sequences were analyzed using the BLASTn alignment program and the NCBI nucleotide database. An alignment score of ≥95% was considered sufficient for species identification.

All 134 bacterial isolates were evaluated by disk-diffusion test with cefoxitin (30μg). MICs were determined by agar dilution test for linezolid and by broth microdilution test for vancomycin and daptomycin according to CLSI M07-A09 and the interpretive criteria described in CLSI M100-S24[9,10]. *Staphylococcus aureus* ATCC 29213 and *Enterococcus faecalis* were used as control strains. The presence of *mecA* gene was evaluated for PCR, where a genomic DNA from each of the isolates was amplified with the primers *mecA*-1 (5′→3′ CTCAGGTACTGCTATCCACC) and *mecA*-2 (5′→3′ CACTTGGTATATCTTCACC) and the product was detected as a 310-bp fragment as previously described[11,12].

The agreement between the results of the different techniques used in this study was determined by statistical analyses, using the Kappa Cohen (κ) statistical method with the criteria outlined by Landis & Koch (1977): poor agreement (kappa index < 0), slight agreement (0.01-0.2), reasonable agreement (0.21-0.40), moderate agreement (0.41-0.60), substantial agreement (0.61-0.80), and almost perfect agreement (0.81-1.00)[13].

The species most frequently identified by *tuf* gene sequencing were *S. epidermidis* (n=67, 50%), *S. haemolyticus* (n=39, 29.1%), and *S. hominis* (n=15, 11.2%) followed by *S. capitis, S. warneri*, *and S. lentus* (9.7%; **Table 1**). The results of MALDI-TOF analysis showed 100% agreement with those of *tuf* sequencing for all strains. The Vitek® 2 Compact and conventional methods exhibited identification discrepancies compared to the standard method and incorrectly identified 9.7% and 23.2% of the bacterial isolates, respectively (**Table 2**). The conventional method could not properly distinguish all species included in this study such as *S. caprae*, and *S. simulans* and was not able to identified *S. capitis* (**Table 1** and **Table 2**). The kappa index for *tuf* gene sequencing and the conventional phenotyping, Vitek® 2 Compact, and MALDI-TOF MS methods was 0.63, 0.85, and 1.00, respectively (data not shown). The susceptibility profile of the bacterial isolates was 92.5% (124/134) resistance to oxacillin. In addition, the *mecA* gene was not detected in two strains (1.5%). In addition, all sample strains were sensitive to daptomycin, linezolid, and vancomycin.

Species identification of CoNS is still a challenge for most clinical laboratories; the results are often unsatisfactory, unreliable, and irreproducible. The conventional and Vitek® 2 Compact methods have limitations in distinguishing between CoNS; however, genotypic techniques, while accurate, are costly and require specialized professionals and equipment that are not available in clinical laboratories. Vitek® 2 Compact exhibited better results than the conventional phenotypic test and could provide a more rapid alternative for improving the laboratory workflow, despite a few discrepancies, which have been observed by other authors as well[4]. A number of previous studies support the use of MALDI-TOF MS analysis. Loonen et al., compared Vitek® 2, gene sequencing identification (*tuf* gene), and MALDI-TOF MS using 142 clinical CoNS isolates; their results indicate that MALDI-TOF showed the best results, identifying 92.3% (131/142) of the isolates[1]. Dubois et al. Carpaij et al. compared *tuf* gene sequencing and MALDI-TOF MS using clinical CoNS isolates and reference strains and showed that MALDI-TOF is an accurate method for distinguishing between species (99.3% and 100% accuracy, respectively)[14,15]. Identification by MALDI-TOF MS represents an innovation for microbiological diagnosis allowing differentiation between a wide variety of bacterial species with

TABLE 1: Summary of results of CoNS clinical isolate species identification using different methods.

Microorganism	*tuf* gene	MALDI-TOF	Vitek® 2	Conventional phenotyping
	strains identified by indicated method [n (%)]			
S. capitis	10 (7.5)	10 (7.5)	9 (6.7)	-
S. caprae	-	-	-	1 (0.7)
S. epidermidis	67 (50.0)	67 (50.0)	59 (44.0)	84 (62.7)
S. haemolyticus	39 (29.1)	39 (29.1)	40 (30.0)	38 (28.4)
S. hominis	15 (11.2)	15 (11.2)	20 (14,9)	6 (4.5)
S. lentus	1 (0.7)	1 (0.7)	1 (0,7)	-
S. simulans	-	-	-	1 (0.7)
S. warneri	2 (1.5)	2 (1.5)	5 (3.7)	4 (3.0)

CoNS: coagulase-negative staphylococci**; MALDI-TOF:** matrix-assisted laser desorption/ionization time-of-flight; ***S.***: *Staphylococcus.*

TABLE 2: Performance of methods used to identify species of CoNS clinical isolates.

Microorganism	Strains identified by the indicated method (n)						
	tuf gene	MALDI-TOF		Vitek® 2		Conventional phenotyping	
		correct	incorrect	correct	incorrect	correct	incorrect
S. capitis	10	10	-	9	1	-	10
S. epidermidis	67	67	-	58	9	63	4
S. haemolyticus	39	39	-	36	3	35	4
S. hominis	15	15	-	15	-	3	12
S. lentus	1	1	-	1	-	-	1
S. warneri	2	2	-	2	-	2	-
Total (%)	**134 (100.0)**	**134 (100.0)**	**-**	**121 (90.3)**	**13 (9.7)**	**103 (76.8)**	**31 (23.2)**

CoNS: coagulase-negative staphylococci*;* **MALDI-TOF:** matrix-assisted laser desorption/ionization time-of-flight; ***S.****: Staphylococcus.*

high accuracy, efficiency, and speed. In this study, the results of MALDI-TOF MS showed high agreement (100%) with those of *tuf* gene sequencing (**Table 1** and **Table 2**), indicating that MALDI-TOF MS is a promising and cost-effective alternative method for identifying CoNS in clinical microbiology settings. Studies with a larger number of isolates should be performed with a greater variety of species of CoNS, in order to confirm our findings for the purpose of early diagnosis and guidance of antibiotic therapy of infections caused by CoNS.

Conflict of interest

The authors declare that there is no conflict of interest.

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